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A Multicentre European Evaluation of the Kodak EKTACHEM GLU/BUN[1]) Analyzer Using NCCLS[2]) Guidelines and Other Approaches

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Summary: This paper describes the field performance of the Kodak EKTACHEM GLU/BUN Analyzer for glucose and urea. NCCLS protocols PSEP-2, 3 and 4 were used which enable manufacturers to establish performance claims concerning the precision and accuracy of an analytical system. This multicentre trial used four analysers in four European countries, United Kingdom, France, West Germany and Italy to assess within and between laboratory performances.

Freeze dried control materials were used for the performance check experiment PSEP-2 and for the replication experiment PSEP-3. The replication study, although very time consuming, was straight forward to undertake and the results were comparable between centres. Compliance with the protocol for comparison of methods experiment PSEP-4 in which laboratories used their own hospital patient samples was more difficult. The problems with obtaining suitable samples and the performance of comparative methods are discussed in detail.

Multizentrische europäische Prüfung des Kodak EKTACHEM GLU/BUN[1])-Analyzers nach NCCLS[2])-Richtlinien und ähnlichen Verfahren

Zusammenfassung: Die Leistung des Kodak EKTACHEM GLU/BUN[1])-Analyzers für Glucose und Harnstoff im täglichen Einsatz wird beschrieben. Als Richtlinien für die Prüfung wurden die NCCLS[2])-Protokolle PSEP-2, 3 und 4, die den Herstellern zur Ermittlung von Leistungsbereichen von analytischen Systemen hinsichtlich Präzision und Richtigkeit dienen, angewandt. Zur Ermittlung der Leistungen innerhalb und zwischen Laboratorien umfaßte die multizentrische Studie vier Geräte in vier europäischen Ländern: Großbritannien, Frankreich, Deutschland und Italien.

Lyophilisierte Kontrollmaterialien wurden für den Versuch zur Prüfung der Leistung (PSEP-2) und den Wiedergabeversuch (PSEP-3) benutzt. Die Wiedergabestudie war, obwohl zeitaufwendig, einfach durchzuführen und ergab zwischen den Zentren vergleichbare Ergebnisse. Übereinstimmung mit dem Protokoll für den Methodenvergleichs-Versuch PSEP-4, für den die Laboratorien Proben ihrer eigenen Krankenhauspatienten einsetzten, war schwieriger zu erzielen. Die Problematik, geeignete Proben zu erhalten und die Leistung von Methoden vergleichend zu untersuchen, wird ausführlich erörtert.

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[1]) The abbreviation GLU is used here for glucose (!), BUN = blood urea nitrogen.

[2]) NCCLS = National Committee for Clinical Laboratory Standards (U.S.A.).

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Introduction

The Kodak EKTACHEM analytical System (1, 2) has recently been introduced into the field of clinical chemistry and Kodak EKTACHEM GLU/BUN Analyzers for glucose and urea analyses are now in routine use in a number of hospitals in the United States of America.

As part of the programme for testing the field performance of the analytical system, four hospital laboratories in the United Kingdom, France, West Germany and Italy used four separate analysers run according to the manufacturers "Operators Manual" (3) over the same period of time to carry out studies using the National Committee for Clinical Laboratory Standards (NCCLS) Protocol for Establishing Performance Claims for Clinical Chemistry Methods (PSEP-2, 3 and 4) (4).

A previous evaluation of the Kodak EKTACHEM products has been published (5). The earlier NCCLS protocol PSEP-1 was available at that time but the study was not on a multicentre basis. In this study the same materials and protocols were used (apart from patient samples) in all the laboratories and this provided a unique opportunity to assess within and between laboratory performance of Kodak EKTACHEM products and to obtain practical experience using the protocol. Since it is essential that manufacturers work jointly with hospitals in the use of these protocols to establish performance claims we have included comments on their practicability.

Materials and Methods

NCCLS protocol for establishing Performance Claims for Clinical Chemistry methods

The protocol is in three sections (4). The sections and the materials used are described below.

1. Performance Check Experiment PSEP-2

The protocol describes the control material to be used and suggests that the materials selected should simulate the characteristics of human sera as closely as possible. HIGH, MID and LOW concentrations should contain where possible analytes at concentrations near the top of the linear range of the method, near the middle of the range for healthy people and near the bottom of the linear range of the method. Construction of control charts is described and these were established for the test methods on the four sites and used throughout subsequent evaluation experiments. Triplicate analyses of the three levels were run on forty occasions during the familiarisation period. The control sera used were lyophilised human material provided by Kodak for the LOW and the HIGH-levels and Wellcomtrol II (Wellcome Reagents Ltd., Kent, U.K.) for the MID level. This data was used to construct mean and range charts. The mean charts consist of a grand mean with upper and lower control limits at ± 3 standard deviations. Range is defined as the difference between the highest and lowest of triplicate readings. The overall mean range is calculated with a control limit set at 2.57 times the mean range.

2. Replication Experiment PSEP-3

The criteria for choice of material at HIGH, MID and LOW levels is as described for PSEP-2. Nine sample sets of HIGH, MID and LOW levels are run after the performance check samples. The sample sets for the maxi, midi and mini experiments are shown in table 1. The order of the nine sample sets are chosen from random permutations provided in the protocol. The replication (imprecision) protocol specifies a period of twenty days and a total of forty analytical runs. The midi version was chosen because it detected carryover effects and provided estimates of within run, between run within day, between runs between day and total imprecision. For each concentration level studied two different estimates of within run and total imprecision are required for presentation of performance claims. The first, designated point estimate, is the actual standard deviation observed in the experiment performed and the second, designated tolerance limit, represents the upper limit that with 95% confidence will contain the estimate of standard deviation from 99% of all similar experiments.

Tab. 1. Sample sets for Replication Experiments PSEP-3. H = high, M = medium, L = low concentration.

Sample subset number	Sample number 1	2	3	4
1	H	H	H	H
2	M	H	H	H
3	L	H	H	H
4	H	M	M	M
5	M	M	M	M
6	L	M	M	M
7	H	L	L	L
8	M	L	L	L
9	L	L	L	L
	MINI	MIDI		
	MAXI			

The replication experiment used the three controls from the performance check period together with Pathonorm L (Nygaard, BDH Poole, England) Wellcomtrol I (Wellcome Reagents Ltd, Beckenham, England) and GEO A632 (General Diagnostics, New Jersey, USA).

3. Comparison of Methods Experiment PSEP-4

In this experiment patient specimens are analysed by both the test method and a comparative analytical method. A suggested concentration distribution of specimens for a number of analytes is given in the protocol.

Table 2 gives the suggested distribution and the actual distribution used for urea and glucose specimens. The comparative methods used by each laboratory, the type of the specimens collected, storage conditions and individual modifications to the protocol are given in table 3.

Four sets of data have been used for regression analysis (tabs. 6 and 7). Set (A) includes results from all specimens; set (B) excluded specimens firstly, if the mean of any duplicate by the Kodak EKTACHEM products was outside the dynamic range specified in the operators manual (3) that is for glucose 1.11–33.3 mmol/l and urea 0.71–42.8 mmol/l. Secondly, if the mean of any duplicate by the comparative method was outside the manufacturers quoted dynamic range.

Set (C) is prepared by using the standard error about the regression line (s_{yx}) calculated from data set (B) to apply

Tab. 2. Percentage distribution of patient specimens (PSEP-4).

Glucose

		A	B	C	D	E
Analyte range mmol/l		< 2.8	2.9–6.1	6.2–8.3	8.4–13.8	> 13.8
Suggested % distribution		10	40	30	10	10
Laboratory site	N =					
U.K.	200	10	40	20	10	20
France	364	5	58	23	13	1
Germany	170	1	57	20	15	7
Italy	111	10	39	29	10	12

Urea

		A	B	C	D	E
Analyte range mmol/l		< 5.3	5.4–8.9	9.0–17.9	18.0–35.6	> 35.6
Suggested % distribution		20	40	20	10	10
Laboratory site	N =					
U.K.	200	20	40	20	10	10
France	355	27	35	26	11	1
Germany	170	43	33	14	6	4
Italy	121	20	40	16	12	12

the test for outliers whereby up to three pairs showing a difference of greater than 3.5 times s_{yx} can be excluded before the final regression analysis. Set D gives the analysis of Wellcome sera.

The regression statistics obtained on data set (C) are used to calculate average bias (yc–xc, where yc is the test method value at medical decision concentration xc) at different medical decision concentrations. Tolerance limits are calculated for yc so that there is a 99% probability that 95% of the samples are included within the upper and lower limits and total error is estimated as the absolute value of the largest difference between the tolerance limits and xc. It is recommended that the tolerance limits and total error be calculated only for medical decision concentrations closest to the mean of the comparative method results.

It has been recommended that the linear regression procedures should be restricted to those cases where the correlation coefficient (r) exceeds 0.99 (6) or that the range of values used is adequate when the standard deviation of the comparative method values (SD_x) is greater than seven time (s_{yx}) (7). Values for both r and SD_x/s_{yx} are included with regression statistics.

Wellcome Group Quality Control Programme

The multi-level lyophilised material available from the programme was used, firstly to obtain information on the performance of comparative methods in each laboratory relative to that obtained by previous participants in the scheme, secondly to provide information on the performance of the EKTACHEM analyzer from laboratory to laboratory, and thirdly to give comparison data additional to that from patient specimens.

Samples previously sent out by the scheme from 16 October 1978–26 March 1979 were analysed by the comparative and Kodak methods at each site. Two duplicate sets of twelve lyophilised bovine sera were provided and a sample for analysis taken from each of the twenty-four bottles after reconstitution, giving twelve duplicate analyses for each analyte.

Analysis of the twelve samples by laboratories participating in the scheme is normally spread over a six month period and the analysis of results returned includes the overall mean for each analyte, that is the mean of all results returned, with results greater than three standard deviations from the mean excluded, and method means which represent the mean of all results from laboratories with a particular method classification. The overall mean values in the samples used ranged from 3.40 to 13.77 mmol/l for glucose and for urea from 5.21 to 23.61 mmol/l. The material in the scheme came from four pools. Different amounts of serum are dispensed to provide each sample and as an identical volume of fluid is recommended for reconstitution this results in different analyte concentrations.

Tab. 3. Procedures used in the Comparison of Methods Experiment.

Laboratory site	Comparative Methods[1] Glucose	Urea	Patient specimen type	Procedure
U.K.	Glucose oxidase[2])/AA1	Diacetyl monoxime[2])/AA1	Fluoride oxalate plasma (glucose), serum (urea)	Specimens pre-selected, frozen, rerun duplicates, one run/day
France	Glucose oxidase/SMA6 Hexokinase/GEMSAEC	Diacetyl monoxime/SMA6	Lithium heparin plasma	Fresh specimens, no duplicates, one run/day
Germany	Hexokinase/SMA12	Diacetyl monoxime/SMA12	Serum	Fresh specimens, replicate 1, a.m. run, replicate 2, p.m. run
Italy	Hexokinase/ACA	Urease-glutamate dehydrogenase/ACA	Serum	Specimens pre-selected, frozen, rerun duplicates in two runs/day

[1]) AA1/Technicon AutoAnalyser Mk. 1
SMA/Technicon Sequential Multichannel Analyser
ACA/Dupont Automatic Clinical Analyser
GEMSAEC/Centrifugal Analyser

[2]) Samples > 20 mmol/l diluted

Results

NCCLS Protocol

1. Replication Experiment (PSEP-3)

Tables 4 and 5 give the analysis of variance (ANOVA) results for the replication experiment. These tables include the mean value (mmol/l) of the concentration level studied, the point estimates of standard deviation and degrees of freedom (df). Tolerance limit estimates of standard deviation are given for within run and total imprecision.

2. Comparison of Methods Experiment (PSEP-4)

Tables 6 and 7 give the regression statistics together with correlation coefficient and the ratio SD_x/s_{yx} for patient samples used in the comparison of methods experiment. The preparation of data sets A, B and C is described in the Materials and Methods section. Regression statistics from data sets A and C were used to calculate the accuracy performance claims given in table 8.

Tab. 4. Statistical analysis (ANOVA) of glucose data (with carryover) from Replication Experiment PSEP-3.

		Within run		Between runs/ within day		Between runs/ between day		Total		
Pathonorm L	Mean (mmol/l)	(df)	S.D.	Tolerance limits	(df)	S.D.	(df)	S.D.	(df)	S.D.	Tolerance limits
U.K.	1.470	(80)	0.0253	0.0391	(20)	0.0165	(19)	0.0683	(119)	0.0747	0.1067
France	1.473	(74)	0.0384	0.0603	(19)	0.0058	(18)	0.1033	(111)	0.1092	0.1579
Germany	1.419	(79)	0.0557	0.0862	(20)	0.0187	(19)	0.0982	(118)	0.1145	0.1638
Italy	1.446	(77)	0.0146	0.0227	(19)	0.0532	(19)	0.0868	(115)	0.1029	0.1479
Wellcomtrol I											
U.K.	6.293	(80)	0.1296	0.2001	(20)	0.0000	(19)	0.1158	(119)	0.1712	0.2445
France	6.320	(75)	0.0695	0.1088	(19)	0.1063	(18)	0.0655	(112)	0.1492	0.2155
Germany	6.301	(78)	0.0552	0.0857	(20)	0.0733	(19)	0.1311	(117)	0.1600	0.2296
Italy	6.194	(78)	0.0400	0.0621	(19)	0.0871	(19)	0.0522	(116)	0.1092	0.1567
Geo A632											
U.K.	26.063	(80)	0.1579	0.2438	(20)	0.1200	(19)	0.4091	(119)	0.4547	0.6496
France	26.855	(71)	0.5124	0.8149	(19)	0.5919	(18)	0.0724	(108)	0.7862	1.4241
Germany	26.148	(76)	0.1902	0.2969	(20)	0.1014	(19)	0.4360	(115)	0.4863	0.6991
Italy	25.883	(77)	0.1376	0.2143	(19)	0.2922	(19)	0.1749	(115)	0.3673	0.5280

Abbreviations used: (df) degrees of freedom, S.D. point estimate of standard deviation.

Tab. 5. Statistical analysis (ANOVA) of urea data (with carryover) from Replication Experiment PSEP-3.

		Within run		Between runs/ within day		Between runs/ between day		Total		
Pathonorm L	Mean (mmol/l)	(df)	S.D.	Tolerance limits	(df)	S.D.	(df)	S.D.	(df)	S.D.	Tolerance limits
U.K.	2.956	(80)	0.0327	0.0505	(20)	0.0194	(19)	0.1152	(119)	0.1214	0.1734
France	2.927	(74)	0.0472	0.0741	(19)	0.0294	(18)	0.0932	(111)	0.1085	0.1569
Germany	2.936	(79)	0.0327	0.0506	(20)	0.0216	(19)	0.0994	(118)	0.1068	0.1536
Italy	2.927	(77)	0.0390	0.0607	(19)	0.0597	(19)	0.1171	(115)	0.1371	0.1971
Wellcomtrol I											
U.K.	9.069	(80)	0.1842	0.2845	(20)	0.1123	(19)	0.1201	(119)	0.2469	0.3527
France	9.020	(74)	0.1442	0.2265	(19)	0.1198	(18)	0.1577	(111)	0.2449	0.3542
Germany	9.151	(78)	0.1016	0.2004	(20)	0.0565	(19)	0.1502	(117)	0.1899	0.2721
Italy	9.094	(78)	0.0938	0.1456	(19)	0.1290	(19)	0.1200	(116)	0.1994	0.2862
Geo A632											
U.K.	31.777	(80)	0.4856	0.7499	(20)	0.1289	(19)	0.2085	(119)	0.5439	0.7769
France	32.000	(70)	1.0203	1.6227	(19)	0.2928	(18)	0.7078	(107)	1.2758	1.8584
Germany	32.055	(76)	0.3459	0.5400	(20)	0.1648	(19)	0.4423	(115)	0.5851	0.8411
Italy	32.232	(77)	0.3386	0.5273	(19)	0.1900	(19)	0.3582	(115)	0.5283	0.7595

Abbreviations used: (df) degrees of freedom, S.D. point estimate of standard deviation.

Tab. 6. Regression analysis of data for glucose Comparative Methods (x) and Kodak EKTACHEM (y) for patient samples and Wellcome group quality control programme.

Laboratory site	Data set[1])	Comparative method[2])	N	Slope[3])	SD slope	Intercept[3])	SD intercept	s_{yx}	r	$\frac{SD_x}{s_{yx}}$	No. of pairs with difference > 3.5 s_{yx}
U.K.	A	Glucose	200	0.9955	0.0042	-0.1777*	0.0461	0.3973	0.9983	17.0	2
	B	oxidase	178	1.0114	0.0062	-0.2764*	0.0525	0.3489	0.9966	12.0	1
	C	AAI	177	1.0048	0.0061	-0.2381*	0.0504	0.3304	0.9968	12.3	0
	D		12	0.9875	0.0157	-0.0882	0.1452	0.2218	0.9987	19.2	0
France	A	Glucose	362	0.9914	0.0067	0.2352*	0.0440	0.3534	0.9919	7.9	0
	B	oxidase	362	0.9914	0.0067	0.2352*	0.0440	0.3534	0.9919	7.9	0
	C	SMA-6	362	0.9914	0.0067	0.2352*	0.0440	0.3534	0.9919	7.9	0
	D		12	1.0008	0.0096	-0.1105	0.0884	0.1336	0.9995	31.4	0
	A	Hexo-	353	1.0243*	0.0067	-0.0672	0.0446	0.3392	0.9925	7.9	3
	B	kinase	350	1.0305*	0.0068	-0.1103	0.0440	0.3057	0.9924	7.8	0
	C	GEM-	350	1.0305*	0.0068	-0.1103	0.0440	0.3057	0.9924	7.8	0
	D	SAEC	–	–	–	–	–	–	–	–	–
Germany	A	Hexo-	170	1.0207*	0.0050	-0.1103*	0.0448	0.3129	0.9980	15.2	2
	B	kinase	168	1.0327*	0.0047	-0.1584*	0.0403	0.2657	0.9983	16.4	3
	C	SMA-12	165	1.0413*	0.0041	-0.2012*	0.0345	0.2242	0.9987	18.9	3
	D		12	0.9840	0.0145	0.0373	0.1331	0.2039	0.9989	20.8	0
Italy	A	Hexo-	111	0.9400*	0.0049	-0.1231*	0.0464	0.2818	0.9985	19.5	1
	B	kinase	109	0.9432*	0.0061	-0.1435*	0.0522	0.2827	0.9977	15.6	1
	C	ACA	108	0.9417*	0.0057	-0.1226*	0.0491	0.2643	0.9980	16.8	0
	D		12	0.9545*	0.0143	-0.4163*	0.1388	0.2022	0.9989	21.1	0

[1]) See Materials and Methods for explanations.
[2]) AA1/Technicon AutoAnalyser Mk. 1
SMA/Technical Sequential Multichannel Analyser
ACA/Dupont Automatic Clinical Analyser
GEMSAEC/Centrifugal Analyser
[3]) Asterisk denotes significant difference from slope of 1.00 or zero intercept (95% confidence limits).

Tab. 7. Regression analysis of data for urea Comparative Methods (x) and Kodak EKTACHEM (y) for patient samples and Wellcome group quality control programme.

Laboratory site	Data set[1])	Comparative method[2])	N	Slope[3])	SD slope	Intercept[3])	SD intercept	s_{yx}	r	$\frac{SD_x}{s_{yx}}$	No. of pairs with difference > 3.5 s_{yx}
U.K.	A	Diacetyl	200	0.8968*	0.0062	0.1792	0.0969	0.9610	0.9967	13.7	2
	B	mon-	162	0.9681*	0.0121	-0.4024*	0.1029	0.5357	0.9877	6.5	0
	C	oxime/	162	0.9681*	0.0121	-0.4024*	0.1029	0.5357	0.9877	6.5	0
	D	AAI	12	0.9525	0.0286	-0.0885	0.4788	0.6626	0.9955	10.5	0
France	A	Diacetyl	355	1.0466*	0.0052	-0.5025*	0.0611	0.6659	0.9956	10.2	7
	B	mon-	354	1.0414*	0.0053	-0.4601*	0.0608	0.6519	0.9955	10.1	6
	C	oxime/	348	1.0346*	0.0046	-0.3961*	0.0511	0.5351	0.9966	11.7	2
	D	SMA6	12	0.9131*	0.0140	-0.2531	0.2334	0.3241	0.9988	21.5	0
Germany	A	Diacetyl	170	0.9816*	0.0049	0.2691*	0.0615	0.5669	0.9979	15.7	2
	B	mon-	167	1.0019	0.0046	0.1440*	0.0504	0.4337	0.9982	16.8	2
	C	oxime/	165	1.0026	0.0040	0.1378*	0.0409	0.3413	0.9987	19.7	0
	D	SMA12	12	0.9501*	0.0171	-0.0432	0.2726	0.3862	0.9984	17.7	0
Italy	A	Urease-	121	1.0071	0.0051	-0.4353*	0.0898	0.6517	0.9985	18.0	2
	B	glutamate	121	1.0071	0.0051	-0.4353*	0.0898	0.6517	0.9985	18.0	2
	C	dehydro-	119	1.0069	0.0049	-0.4379*	0.0758	0.5446	0.9989	20.8	1
	D	genase/ACA	12	0.9518*	0.0196	-0.2932	0.3179	0.4472	0.9979	15.4	0

[1]) See materials and methods for explanation.
[2]) AA1/Technicon AutoAnalyser Mk. 1
SMA/Technical Sequential Multichannel Analyser
ACA/Dupont Automatic Clinical Analyser
GEMSAEC/Centrifugal Analyser
[3]) Asterisk denotes significant difference from slope of 1.00 or zero intercept (95% confidence limits).

Wellcome Group Quality Control Programme

Table 9 gives the regression statistics which indicate the performance of the comparative methods (y) in each laboratory against the appropriate method mean for each laboratory.

Tables 10 and 11 show the regression statistics for the performance of the EKTACHEM analyzer from laboratory to laboratory and tables 6 and 7 give in data sets D additional information from each laboratory for the comparative method with the EKTACHEM analyzer. No exclusion criteria were applied to the results in this section as the number of samples was small. The ratio SD_x/s_{yx} in every case was well in excess of 7.0 and no pairs showed a difference in excess of 3.5 times s_{yx}.

Tab. 8. Accuracy Performance Claims at selected medical decisions. Concentrations for glucose and urea.

	Data set[2])	Medical decision level xc	$\bar{x}$	$\bar{Y}c$	Average bias yc-xc	Tolerance limits	Estimate of total error
Glucose							
U.K. (Glucose oxidase/AA1[1]))	A	6.60	8.77	6.55	- 0.05	5.70- 7.40	0.90
	C	6.60	7.18	6.39	- 0.21	5.68- 7.10	0.50
France (Glucose oxidase/SMA6)	A	6.60	5.96	6.78	0.18	6.02- 7.54	0.94
	C	6.60	5.96	6.78	0.18	6.02- 7.54	0.94
France (Hexokinase/GEMSAEC)	A	6.60	6.07	6.69	0.09	5.96- 7.42	0.82
	C	6.60	5.98	6.69	0.09	6.03- 7.35	0.75
Germany (Hexokinase/SMA-12)	A	6.60	7.35	6.63	0.03	5.95- 7.31	0.71
	C	6.60	6.94	6.67	0.07	6.19- 7.15	0.55
Italy (Hexokinase/ACA)	A	6.60	7.28	6.08	- 0.52	5.45- 6.71	1.15
	C	6.60	7.31	6.09	- 0.52	5.50- 6.68	1.10
Urea							
U.K. (Diacetyl monoxime/AA1)	A	9.60	13.34	8.79	- 0.81	6.73-10.85	2.87
	C	9.60	7.76	8.89	- 0.71	7.74-10.04	1.86
France (Diacetyl monoxime/SMA6)	A	9.60	9.44	9.54	- 0.06	8.11-10.97	1.49
	C	9.60	9.24	9.54	- 0.06	8.39-10.69	1.09
Germany (Diacetyl monoxime/SMA-12)	A	9.60	8.15	9.69	0.09	8.47-10.91	1.31
	C	9.60	7.83	9.76	0.16	9.02-10.50	0.90
Italy (Urease-glutamate dehydrogenase/ACA)	A	9.60	13.33	9.23	- 0.37	7.80-10.66	1.80
	C	9.60	12.88	9.23	- 0.37	8.04-10.42	1.56

[1]) AA1/Technicon AutoAnalyser Mk. 1
SMA/Technicon Sequential Multichannel Analyer
ACA/Dupont Automatic Clinical Analyser
GEMSAEC/Centrifugal Analyser
[2]) See text for explanation.

Tab. 9. Comparative Methods results (y axis) compared with the Appropriate Method Mean (x axis) in the Wellcome group quality control programme.

Laboratory	Method[1])	N	Slope[2])	S.D. slope	Intercept[2])	S.D. intercept	s_{yx}	r	$\frac{SD_x}{s_{yx}}$
U.K.	Glucose oxidase/AA1	12	1.0054	0.0140	- 0.0423	0.1294	0.1968	0.9990	21.5
France	Glucose oxidase/SMA-6	12	0.9396*	0.0114	0.1772	0.1100	0.1690	0.9993	26.4
Germany	Hexokinase/SMA-12	12	0.9924	0.0084	- 0.1246	0.0782	0.1184	0.9996	36.1
Italy	Hexokinase/ACA	12	0.9991	0.0123	0.4101*	0.1155	0.1747	0.9992	24.4
U.K.	Diacetyl monoxime/AA1	12	1.0205	0.0097	0.2148	0.1565	0.2191	0.9996	31.2
France	Diacetyl monoxime/SMA-6	12	1.0199	0.0058	0.1430	0.1344	0.1881	0.9997	36.3
Germany	Diacetyl monoxime/SMA-12		0.9987	0.0113	- 0.2044	0.1822	0.2549	0.9994	26.8
Italy	Urease-glutamate dehydrogenase/ ACA	12	1.0445*	0.0152	- 0.3220	0.2407	0.3322	0.9989	19.8

[1]) AA1/Technicon AutoAnalyser Mk. 1
SMA/Technicon Sequential Multichannel Analyser
ACA/Dupont Automatic Clinical Analyser
GEMSAEC/Centrifugal Analyser
[2]) Asterisk indicates significantly different from 1.00 or zero at 95% confidence limits.

Tab. 10. Kodak/Kodak comparisons for glucose using Wellcome group quality control programme material.

x	y	N	Slope[1])	S.D. slope	Intercept	S.D. intercept	s_{yx}	r	$\frac{SD_x}{s_{yx}}$
U.K.	France	12	0.9984	0.0162	0.1123	0.1467	0.2264	0.9987	18.6
	Germany	12	0.9897	0.0236	0.1263	0.2136	0.3297	0.9972	12.8
	Italy	12	0.9684	0.0161	0.1669	0.1453	0.2242	0.9986	18.8
France	U.K.	12	0.9990	0.0162	-0.0908	0.1483	0.2265	0.9987	18.6
	Germany	12	0.9922	0.0117	0.0083	0.1067	0.1630	0.9993	25.8
	Italy	12	0.9694*	0.0105	0.0622	0.0956	0.1460	0.9994	28.8
Germany	U.K.	12	1.0046	0.0240	-0.0811	0.2174	0.3321	0.9972	12.6
	France	12	1.0065	0.0119	0.0030	0.1075	0.1641	0.9993	25.4
	Italy	12	0.9761	0.0128	0.0618	0.1165	0.1779	0.9991	23.5
Italy	U.K.	12	1.0298	0.0171	-0.1496	0.1522	0.2312	0.9986	17.6
	France	12	1.0393*	0.0111	-0.0545	0.0991	0.1505	0.9994	27.1
	Germany	12	1.0227	0.0135	-0.0491	0.1199	0.1821	0.9991	22.4

[1]) Asterisk indicates significantly different from 1.00 at 95% confidence limits.

Tab. 11. Kodak/Kodak comparisons for urea using Wellcome group quality control programme material.

x	y	N	Slope[1])	S.D. slope	Intercept	S.D. intercept	s_{yx}	r	$\frac{SD_x}{s_{yx}}$
U.K.	France	12	0.9504	0.0219	0.6159	0.3311	0.4845	0.9974	13.8
	Germany	12	0.9670	0.0299	0.5332	0.4521	0.6616	0.9953	10.1
	Italy	12	0.9810	0.0207	0.3667	0.3142	0.4598	0.9978	14.5
France	U.K.	12	1.0467	0.0241	-0.5724	0.3602	0.5084	0.9974	15.5
	Germany	12	1.0186	0.0144	-0.1088	0.2161	0.3051	0.9990	20.9
	Italy	12	1.0309*	0.0133	-0.2516	0.1985	0.2802	0.9992	22.7
Germany	U.K.	12	1.0243	0.0316	-0.4166	0.4789	0.6810	0.9953	9.5
	France	12	0.9797	0.0139	0.1340	0.2104	0.2992	0.9990	21.7
	Italy	12	1.0104	0.0172	-0.1189	0.2600	0.3698	0.9986	17.6
Italy	U.K.	12	1.0148	0.0215	-0.3111	0.3261	0.4676	0.9978	14.1
	France	12	0.9684*	0.0125	0.2663	0.1894	0.2716	0.9992	24.3
	Germany	12	0.9868	0.0168	0.1571	0.2549	0.3654	0.9986	18.0

[1]) Asterix indicates significantly different from 1.0 at 95% confidence limits.

Discussion

Problems encountered with PSEP-2, 3 and 4

The establishment of performance claims for Clinical Chemical Methods has become a major expense for manufacturers of clinical chemistry systems and a time consuming occupation for skilled laboratory workers.

In these activities, however, there are complex problems, for manufacturers and clinical chemistry laboratories alike and the publication of proposed standards PSEP-2, 3 and 4 by the NCCLS represent an important contribution to progress in this field.

This paper reports some of our experience with these standards and the data derived from our work. The PSEP-2 and 3 standards although time consuming, presented few difficulties in execution at the different sites. However, all sites encountered some difficulties in carrying out the proposed standard for the Comparison of Methods Experiment (PSEP-4). The difficulties related on the one hand to the selection of patient specimens and their analysis according to the protocol and on the other hand to the performance of the comparative methods during the period of study.

The overview of the Comparison of Methods Experiment suggests that "at least 100 fresh patient's specimens should be analysed in duplicate by both the test method and the comparative analytical method. The experiment must cover a period of at least 4 days, which permits a maximum of 25 specimens to be analysed in one day, or it can extend over a longer period of time if that is convenient for the evaluation study". Recommendations for the selection of patients specimens are given and one suggested distribution is shown in table 2.

It is clear that all sites encountered difficulties in selection of specimens and the laboratories in Italy and the U.K. were only able to comply with the suggested distribution by preselecting specimens and freezing them prior to subsequent duplicate analysis (tab. 3). With the exception of the laboratory in France all sites

analysed the specimens in duplicate. The laboratory in France however ran nearly twice the number of specimens as the other laboratories and as a result regression estimates will not be markedly affected. However, only the German laboratory complied with the stipulation that the replicates should be in separate runs. One penalty of not running duplicates is the failure to produce within run estimates of imprecision for human sera for the test and comparative methods. The information is important for evaluation of the comparative method and for its comparison with the test method.

Additionally, these estimates of within run imprecision can be usefully compared with those obtained in the replication experiment using the lyophilised material. If the patient specimens in the German laboratory are replicated between run rather than within run, different estimates of precision are obtained which are not strictly comparable.

The amount of specimen required in order to perform duplicate analysis by the test and comparative method represents a major problem if the test and/or comparative methods require substantial amounts of serum or plasma. The use of this protocol for evaluation of multichannel systems may present special difficulties, although one such evaluation has recently been published (9).

There is a tendency when selecting specimens for analysis to encounter difficulties at the ends of the range. This can lead to the multiple selection of specimens from one patient so that although the number of specimens required is fulfilled the variability represented by those specimens is reduced. If this were to become a major feature of selection then it might result in falsely low estimates of s_{yx} which would markedly improve the accuracy performance claims. It is interesting in this connection to compare the regression statistics for comparison of methods for the bovine material from the Wellcome Scheme (tabs 6 and 7 data sets D) with those obtained on patient specimens (data sets C). Bearing in mind the recognised problems associated with commutability of human specimens and samples from animal sources together with the small number of Wellcome samples used, the majority of estimates of slope and intercept are in good agreement with those obtained with patient specimens. However, the value for s_{yx} in all laboratories for urea and glucose using Wellcome material is markedly lower than the value on patient specimens. This reflects the fact that the Wellcome material is taken from four homogenous pools and the smaller range covered.

If in excess of fifty quality control samples were used in a comparison of methods and commutability were satisfactory the standard deviation of the estimates of slope and intercept would be markedly improved (8) but analysis of lyophilised material from different sources can never replace patient specimens in estimation of the standard error of the regression line.

France used lithium heparin plasma specimens for glucose and urea analyses and the United Kingdom used fluoride oxalate plasma for glucose. Care must be taken to ensure that plasma specimens prepared in this way are obtained from blood specimens which had the recommended amounts of anticoagulant added. High concentrations, resulting from inadequate filling of a specimen container, can adversely affect measurement by a test or comparative method.

The choice and control of the comparative method represents the second major problem in the comparison of methods experiment and whereas the protocol discusses briefly the factors affecting the choice of a comparative or reference method it does not provide guidance as to the control of that method during the period of study. The data presented in table 9 represents an attempt to provide some information about the bias of the comparative methods. The significant (95% confidence limits) positive intercept of 0.4101 in the glucose data from Italy indicates the presence of constant error whereas the glucose data from France and the urea data from Italy indicate a significant proportional error in these methods when compared with their appropriate method means. When an individual laboratory results are compared with method means it must be remembered that the method mean codes used in this instance do not define a group of laboratories using a particular instrument with a particular method but perhaps as in the case of the code for the hexokinase/glucose method it contains on average 114 laboratories using instruments from at least nine different manufacturers some employing discrete analysis and some continuous flow. Therefore caution must be exercised when interpreting such a comparison.

Performance of the Kodak EKTACHEM GLU/BUN Analyzer and preparation of Performance Claims

The Kodak EKTACHEM GLU/BUN analyzer performed well on all sites as judged by estimates of imprecision by using the proposed standard PSEP-3 (tab. 4 and 5), and analysis of data from all laboratories indicates the very small contribution made to the overall variance by the laboratory to laboratory component. The differences in tolerance limits and estimates of total error (tab. 8) obtained from the comparison of methods standard PSEP-4 are influenced by the way in which data is analysed. The performance of the comparative methods is also important and makes a simultaneous multicentre study in evaluation of methods particularly valuable when claims for a product are put forward.

In the preparation of accuracy performance claims it is clear that calculation of bias is dependent on reliable estimates of slope and intercept and that the tolerance

limits are additionally dependent on the standard error about the regression line (s_{yx}). It is interesting in tables 6 and 7 to see that the rule governing the exclusion of outliers can either eliminate or reduce outliers (compare data sets B with C) or because of the redefinition of s_{yx} implicit in creating a new data set can lead to the same numbers of outliers still existing (see German data set B and C for glucose tab. 6). Preparation of data in the manner recommended will sometimes lead to a reduction in the range of samples analysed and additionally the removal of outliers will reduce the value of s_{xy}. For performance claims to be comparable these factors must be taken into account. This effect can be seen by comparing the performance claims based on the whole data set A with those given for the prepared data set C.
The protocol suggested that tolerance limits and total error be calculated only for medical decision concentrations closest to the mean of the comparative method data (x) table 8 shows that for glucose (data sets C) this requirement is reasonably well fulfilled. For the medical decision concentration of 6.6 mmol/l values of x range from 5.96 to 7.31 mmol/l. However for urea the situation is less than satisfactory with mean values of x ranging from 7.76 to 12.88 mmol/l for a medical decision concentration of 9.60 mmol/l. This problem has however already appeared in the literature (9) with medical decision concentrations of 1100 mg/l (6.1 mmol/l) for glucose having tolerance limits and total error quoted when the mean of comparative or reference method was 1670 mg/l (9.3 mmol/l) and 1600 mg/l (8.9 mmol/l) respectively and for a medical decision concentration of 250 mg/l ((8.9 mmol/l) for urea nitrogen with mean of x at 512 mg/l (18.3 mmol/l). It will be necessary to indicate how close is close if performance claims are to be of value. The mean of the comparative method (x) should be given in an accuracy performance claim in order to avoid misunderstanding.

The Wellcome material was also used to provide additional data on EKTACHEM products from site to site (tab. 10 and 11). The estimates of slope were significantly different from 1.00 (95% confidence limits) for both urea and glucose between France and Italy whereas no significant differences from zero were observed for estimates of intercept. These differences are not however clearly reflected in the mean values of material used in the replication study (Tab. 4 and 5).

Conclusions

The proposed standards PSEP-2, 3 and 4 have proved a useful basis on which to establish this multi-centre trial of the Kodak EKTACHEM GLU/BUN analyzer. The difficulties associated particularly with PSEP-4 have been fully discussed. It is suggested that the proposed standard, PSEP-4 would be improved by inclusion of some basic criteria for evaluation of the comparative method against other laboratories in form of methods means. It is in this area that manufacturers are most vulnerable to claims made for or against their products by laboratories using inadequately controlled comparative or reference techniques.

Because of the difficulties associated with obtaining patient samples and the labile nature of some analytes, manufacturers will always require the assistance of clinical chemistry laboratories in the establishment of performance claims, but our experience suggests, that this work should not be undertaken lightly by laboratories and that manufacturers would be advised to assess the resources of any chosen site carefully before proceeding.

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References

1. Curme, H. G., Columbus, R. L., Dappen, G. M., Eder, T. W., Fellows, W. D., Figueras, J., Glover, C. P., Goffe, C. A., Hill, D. E., Lawton, W. H., Muka, E. J., Pinney, J. E., Rand, R. N., Sanford, K. J. & Wu, T. W. (1978) Clin. Chem. *24*, 1335–1342.
2. Spayd, R. W., Bruschi, Barbara, Burdick, B. A., Dappen, G. M., Eikenberry, J. N., Esders, T. W., Figueras, J., Goodhue, C. T., LaRossa, Denise D., Nelson, R. W., Rand, R. N. & Wu, T. W. (1978) Clin. Chem. *24*, 1343–1350.
3. Kodak EKTACHEM Glu/BUN Analyzer – Operator's Manual (Eastman Kodak Co., Rochester, N.Y.).
4. Draft Documents (1978) PSEP-2, PSEP-3 and PSEP-4, Instrument Evaluation Sub-committee, Evaluation Protocols Area, National Committee for Clinical Laboratory Standards, 771 E. Lancaster Avenue, Villanova, PA 19085.
5. Cate IV, J. C., Hedrick, Regina, Taylor, M. & McGlothlin, C. D. (1980) Clin. Chem. *26*, 266–270.
6. Wakkers, P. J. M., Hellendoorn, H. B. A., Op De Weegh, G. J. & Heerspink, W. (1975) Clin. Chim. Acta *64*, 173–184.
7. Westgard, J. O., de Vos, D. J., Hunt, M.R., Quam, E. F., Garber, C. C. & Carey, R. N. (1978) Am. J. Med. Technol. *44*, 552–571.
8. Davis, R. B., Thompson, J. E. & Pardue, H. L. (1978) Clin. Chem. *24*, 611–620.
9. Garber, C. C., Westgard, J. O., Milz, Linda & Larson, F. C. (1979) Clin. Chem. *25*, 1730–1738.

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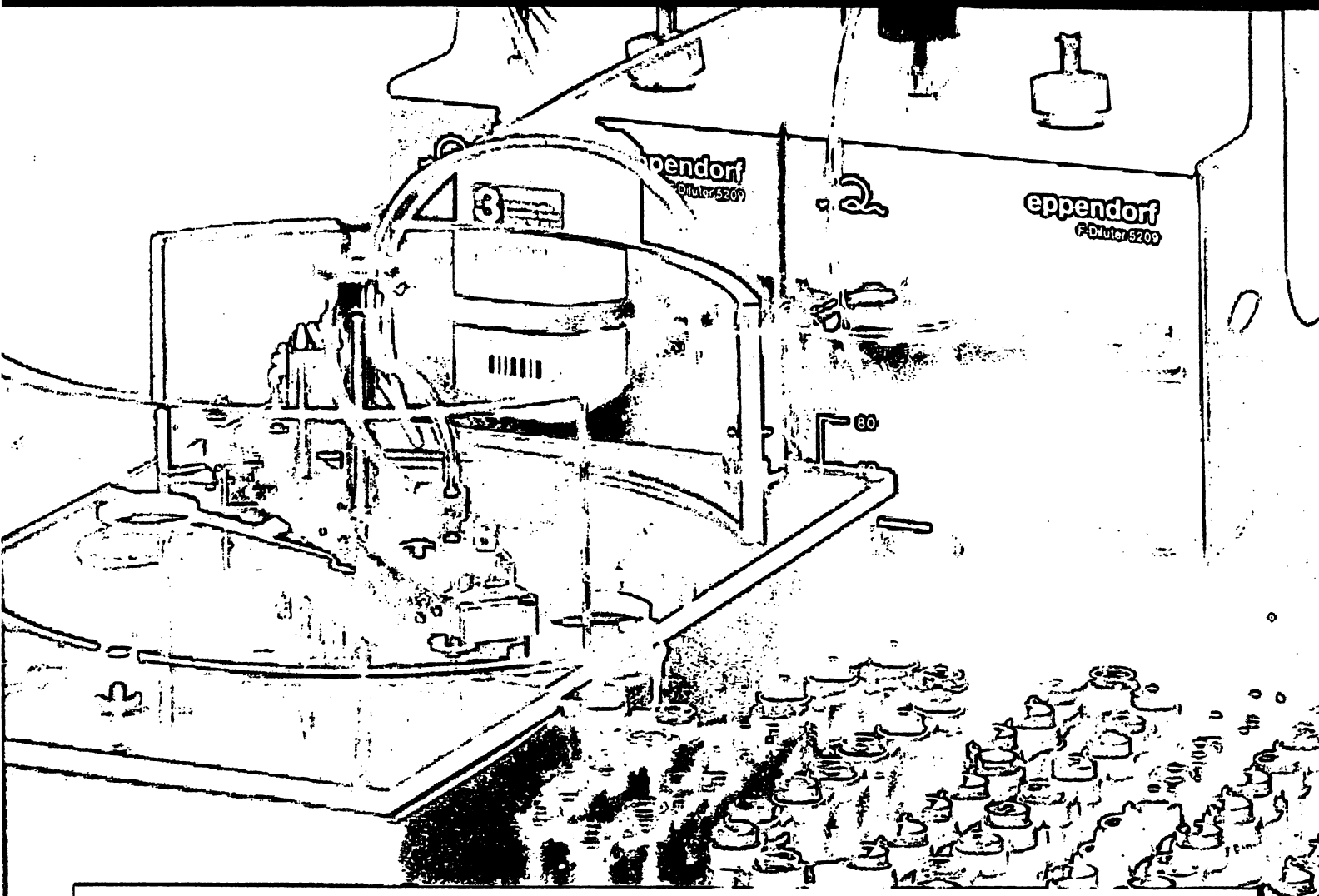

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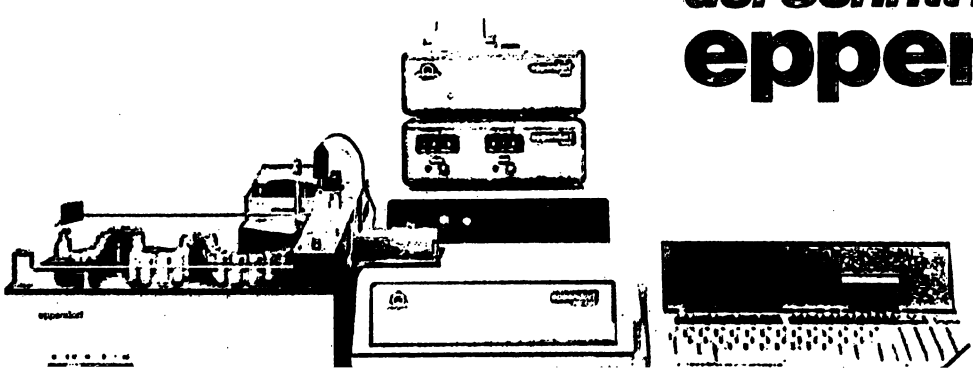

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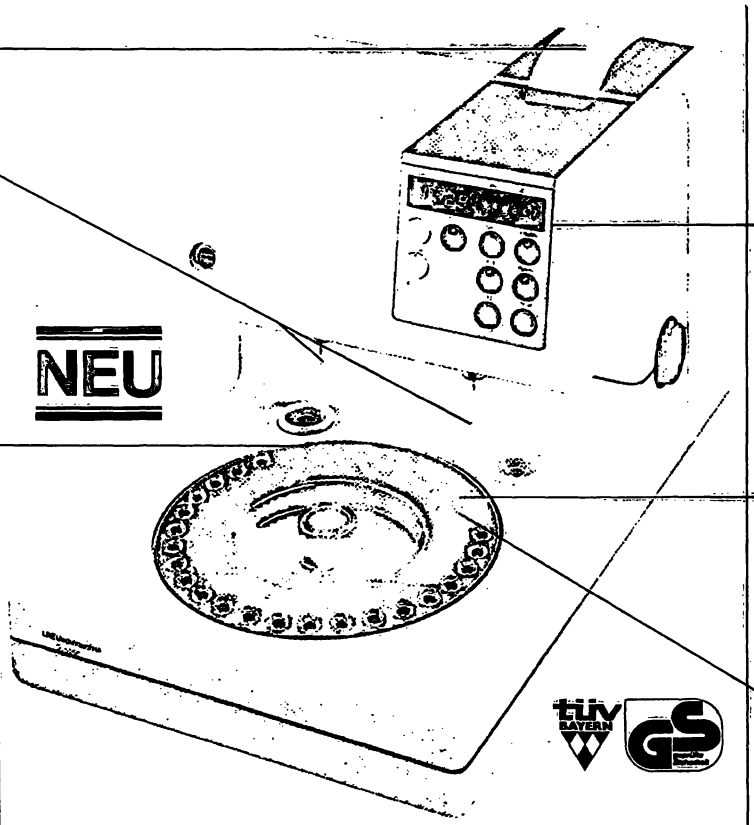

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